PP 2-23

FUNCTIONAL CHARACTERIZATION OF *RHIZOBIUM LEGUMINOSARUM* BV. VICIAE DMERF, A CATION DIFFUSION FACILITATOR SYSTEM INVOLVED IN NICKEL AND COBALT RESISTANCE

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In prokaryotes, nickel is an essential element participating in the structure of enzymes involved in multiple cellular processes. Nickel transport is a challenge for microorganisms since, although essential, high levels of this metal inside the cell are toxic. For this reason, bacteria have developed high-affinity nickel transporters as well as nickel-specific detoxification systems. Ultramafic soils, and soils contaminated with heavy metals are excellent sources of nickel resistant bacteria. Molecular analysis of strains isolated in the habitats has revealed novel genetic systems involved in adaptation to such hostile conditions.

In order to identify genetic systems involved in nickel homeostasis, a random mutagenesis by inserting a Tn5-derivative minitransposon was carried out in *Rhizobium leguminosarum* bv. *viciae* UPM1137, a strain isolated from an ultramafic soil that shows a high level of nickel resistance. 16 mutants unable to grow at high nickel concentrations were isolated. In this work we present the functional and expression analysis of *R. leguminosarum dmeRF* genes, one of the genetic systems identified by this approach.

The DmeRF (Divalent Metal Efflux) system of *R. leguminosarum* is required for nickel and cobalt resistance. DmeF is a transmembrane protein belonging to the Cation Diffusion Facilitator (CDF) transport family, whereas DmeR is a regulator that shows homology to the nickel responsive metalloregulator RcnR. Analysis of the *dmeRF* gene expression by promoter fusions and q-PCR showed that these genes are organized as an operon inducible by nickel and cobalt ions in the culture medium as well as in symbiotic conditions, where DmeR acts as a repressor of its own expression under low nickel conditions.

Sequence analysis of draft genomes from UPM1137 and from several strains isolated from the same ultramafic soil revealed the presence of additional putative nickel detoxification systems not previously identified by random mutagenesis, whose functional characterization is being carried out.